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GENETIC ANALYSIS OF THE PRESENTATION OF MINOR LYMPHOCYTE-STIMULATING DETERMINANTS

II. Differing Non-MHC Control of Super-Stimulatory and More Poorly Stimulatory Mls Phenotypes[1]

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Analysis of the capacity of splenocytes from non-prototypic Mls^a or Mls^c mouse strains to stimulate allogeneic H-2^k-compatible T cells in a primary Mls-defined MLR provided interesting examples of exceptions to the usually stated characterization of Mls^a and Mls^c determinants as highly stimulatory or weakly stimulatory, respectively. Across the Mls^a barrier, MA/My stimulator cells had a significantly reduced capacity to elicit responder proliferation in comparison with prototypic AKR/J or less well studied C58/J, CE/J, or RF/J splenocytes. Across the Mls^c barrier, a gradient of stimulatory ability was observed with RF/J splenocytes being virtually non-stimulatory, prototypic C3H/HeJ splenocytes having an intermediate capacity, and CE/J and C58/J being highly stimulatory presenters of this non-MHC specificity. The differing capacity of each of these H-2^k stimulator cells to elicit unprimed responder cell proliferation across an Mls^a or Mls^c difference correlated with the T cell growth factor activity that was secreted into the MLR supernatants. The super-stimulatory form of Mls^c was expressed in an autosomal dominant fashion by (Mls^c poorly stimulatory × Mls^c super-stimulatory)F$_1$ animals, (BALB.K × C58/J)F$_1$ or (RF/J × CE/J)F$_1$. The segregation of Mls^c stimulatory ability among first backcross and F$_2$ animals derived from the former F$_1$ was compatible with a single non-MHC gene controlling the expression and presentation of the super-stimulatory form of Mls^c. The regulatory nature of this gene was indicated by the observation that F$_1$ animals generated from the Mls^c nonprototypic and poorly stimulatory BALB/c parental strain were self-tolerant to the super-stimulatory form of Mls^c. The existence of an Mls specificity other than a and c was suggested by positive non-MHC MLR responses in certain responder/stimulator cell combinations of Mls prototypic and nonprototypic mouse strains.

It has been known for over 20 years that murine non-MHC-encoded gene products, termed Mls[3] determinants, presented by lymphoid stimulator populations, can trigger naive H-2-compatible allogeneic T cells to proliferate in a primary MLR (1). Recent genetic studies have shown that the Mls system is not comprised of a single locus as originally (2, 3) proposed; at least two unlinked Mls specificities, Mls^a and Mls^c, exist (4). In addition, the Mls^d moiety has been shown to represent the simultaneous expression of both Mls^a and Mls^c determinants (5–7). Some inbred mouse strains, originally typed as Mls^a or Mls^b, have been shown to encode Mls^c (8–11). Further studies also suggest that multiple genes may control the stimulation across an Mls^a (12) or an Mls^c (13–15) barrier.

Interestingly, the expression of certain TCR Vβ gene products is strongly associated with T cell responsiveness to certain Mls gene products (14, 16, 17). Mature thymocytes and peripheral T cells in Mls^a-bearing mice of an appropriate MHC haplotype were deleted of Vβ8.1$^+$ (16) or Vβ6$^+$ (18) TCR whereas these cell types obtained from Mls^c-bearing mice were deleted of Vβ3$^+$ (14, 17, 19) TCR.

However, only the self-deleting Mls^a super-Ag has been considered highly stimulatory, with large numbers of CD4$^+$ (20) T cells from H-2-compatible Mls^a-negative mouse strains precommitted to proliferate to this non-MHC moiety. In contrast, the Mls^c specificity has been routinely regarded as poorly to intermediately stimulatory (2–7, 10, 14, 15, 18, 21–35), with one study also suggesting that the precursor frequency of Mls^c-specific T cells in a heterogeneous T cell population is low (10).

This laboratory has recently shown that multiple non-MHC immune response genes significantly influence the presentation of Mls^c gene products expressed on splenic stimulator cells with permissive class II MHC restriction elements (8). In the present study we extend the latter investigation by the analysis of the primary MLR-stimulatory potential of Mls^a and Mls^c determinants expressed on splenic stimulator cells from poorly studied, nonprototypic, Mls-bearing mouse strains. By obtaining a more

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[3] Abbreviations used in this paper: Mls, minor lymphocyte stimulating; GaMD, goat anti-mouse IgD antibody.

comprehensive understanding of the true variation in Mls^a and Mls^c-stimulatory capacity with nonprototypic mouse strains we attempt: a) to bring experimental scrutiny to the commonly held view regarding differences in MLR-stimulatory potential between Mls^a and Mls^c, b) to probe for additional underlying features of non-MHC genetic regulation of these moieties, and c) to search for heretofore unidentified non-MHC MLR-stimulatory specificities and possible members of the Mls "family" of determinants in poorly studied H-2-compatible responder/stimulator combinations.

MATERIALS AND METHODS

Animals. AKR/J (H-2^k, Mls^a), (AKR/J × DBA/2)F$_1$ (H-2$^{k/d}$, Mls^{a,c}), (BALB/c × DBA/2)F$_1$ (H-2^d, Mls^{a,c}), (BALB/cbyJ × DBA/2)F$_1$ (H-2^d, Mls^{a,c}), B10.BR (H-2^k, Mls^b), B10.D2 (H-2^d, Mls^b), CBA/J (H-2^k, Mls^{a,c}), CE/J (H-2^k, Mls^{a,c}), C3H/HeJ (H-2^k, Mls^c), (C57BL/6 × DBA/2)F$_1$ (H-2$^{b/d}$, Mls^{b,a,c}), C57BL/10Sn (H-2^b, Mls^b), C58/J (H-2^k, Mls^{a,c}), MA/My (H-2^k, Mls^a), RF/J (H-2^k, Mls^{a,c}), and SJL/J (H-2^s, Mls$^?$) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. (AKR/J × BALB/c)F$_1$ (H-2$^{k/d}$, Mls^{a,c}), (AKR/J × B10.D2)F$_1$ (H-2$^{k/d}$, Mls^{a,b}), (AKR/J × C3H)F$_1$ (H-2^k, Mls^{a,c}), (BALB.K × C58)F$_1$ (H-2^k, Mls^{a,c}), (B10.BR × C58)F$_1$ (H-2^k, Mls^{b,a,c}), (B10.BR × RF/J)F$_1$ (H-2^k, Mls^{b,a,c}), (CBA/N × C3H)F$_1$ (H-2^k, Mls^{null,c})F$_1$, and (CBA/N × C58)F$_1$ (H-2^k, Mls^{null,a,c}) mice were bred at the Naval Medical Research Institute with parental breeding stock purchased from The Jackson Laboratory with the exception of BALB.K (H-2^k, Mls^c) mice that were kindly provided by Dr. Florence Rollwagen. Because it is likely that both Mls^a (12) and Mls^c (13–15) represent the products of multiple genes, the original small case letter designations for Mls specificities have been utilized throughout this study as collective symbols for these groups of multigene products. Experiments were performed with 8- to 20-wk-old animals that were maintained in filtered air isolators in the animal colonies of the Naval Medical Research Institute.

MLR. Responder cells for the MLR were obtained by enriching for T cells by passage of splenocytes over a nylon wool column according to the method of Julius et al. (36). Unprimed responder T cells were cultured at density of 3 × 10^5 cells/microtiter well, usually with 1.5 to 9 × 10^5 stimulator cells in a total volume of 0.2 ml. The MLR culture medium consisted of RPMI 1640 (Hazelton Laboratories, Lenexa, KA) with gentamicin (50 μg/ml), L-glutamine (2 mM), HEPES buffer (25 mM), 5% FCS (HyClone, Ogden, UT), and 5 × 10^{-5} M 2-ME. Each MLR was performed with quadruplicate cultures in round bottomed microculture plates (No. 3799; Costar, Cambridge, MA) and was maintained in a humidified atmosphere of 5% CO_2 at 37°C. The culture was harvested after 72 to 108 h onto glass fiber filter paper after a 12-h pulse with 1 μCi (37 kBq)/microtiter well of [^{3}H]TdR (specific activity = 2 Ci/mM, New England Nuclear, Boston, MA). Incorporated [^{3}H]TdR was measured on a Beckman scintillation spectrometer. The results were calculated from uptake of [^{3}H]TdR and are expressed as the arithmetic mean in cpm of quadruplicate cultures. The SE were generally less than 10% of the mean. The χ^2 test was used to compare observed and expected MLR data in the first backcross and F_2 segregation analysis.

Assay of T cell growth factor activity in MLR supernatants. HT-2 cells (4 × 10^{-3}), resuspended in MLR medium (see above), were added to flat bottom microtiter dishes (No. 76-032-05; Linbro, McLean, VA) together with serial dilutions of 48-h supernatants from Mls-defined MLR or known recombinant cytokines, in quadruplicate cultures; the total volume per culture well was 0.1 ml. These microtiter dishes were maintained at 37°C in a humidified atmosphere with 5% CO_2 for 24 h; a pulse of 1 μCi/well of [^{3}H]TdR was added during the last 6 h of culture before harvest. Incorporated [^{3}H]TdR was measured as indicated above. The HT-2 cell line has been reported to be responsive to IL-2, IL-4, and granulocyte-macrophage CSF (37). However, this cell line, after maintenance at this institute for 2 yr, retained responsiveness only to IL-2 and IL-4 as demonstrated with the appropriate recombinant murine cytokine reagents obtained from Genzyme (Boston, MA) (data not shown).

In vivo anti-IgD treatment and in vitro preparation of MLR stimulator cells. Recipient mice were injected i.v. with 100 μg of affinity-purified GaMD in a volume of 0.2 ml, prepared as previously described (38). Twenty-four hours later the spleens were removed and were gently teased in HBSS with 10% FCS, were irradiated with 3000 R (^{137}Cs) after the removal of the RBC with a hypotonic lysing buffer, were washed again in HBSS with 10% FCS, and were resuspended in the MLR medium described above. This laboratory has shown that in vivo (24, 33, 39) or in vitro (39) activation of splenic B cells with GaMD before irradiation substantially enhances the capacity to present both Mls specificities. When mitomycin C (85.549.9; Aldrich Chemical Co., Milwaukee, WI) was used instead of irradiation to prevent normal (non-GaMD-treated) splenic stimulator cells from dividing in the primary MLR, these splenocytes (1 × 10^7/ml) were suspended in HBSS and were exposed to mitomycin C (75 μg/ml) for 30 min at 37°C in the absence of light and were then washed three times before being added to the responder T cells.

T cell depletion of splenocyte populations. Spleens were gently teased in RPMI 1640 plus 10% FCS, depleted of RBC with ammonium chloride lysing buffer, washed twice, and filtered through sterile nylon mesh (HC3-110; Tetco, Elmsford, NY) to remove tissue clumps. The single cell preparation was then suspended in monoclonal anti-Thy-1.2 ascites fluid (NEI-001, New England Nuclear Research Products, Boston, MA) at 1/500 dilution for ½ h at 4°C. The treated cells were then washed twice and resuspended in a 1/8 dilution of rabbit C (ACL 3051, Low-Tox M, Accurate Chemical and Scientific Corp., Westbury, NY) for 45 min at 37°C. The remaining splenoctyes were washed twice and again passed through sterile nylon mesh to remove dead cells and tissue debris before treatment with mitomycin C (see above).

RESULTS

Wide variation in Mls^a- and Mls^c-presenting capacity exists with stimulator cells obtained from different H-2^k-compatible mouse strains. In order to test the commonly accepted belief, based on the examination of certain prototypic mouse strains, that Mls^a and Mls^c determinants were highly or poorly to intermediately stimulatory, respectively, splenocytes from other less well studied H-2^k mouse strains were analyzed for their Mls^a and Mls^c presenting capacity in a primary MLR.

Whether normal (nonactivated) mitomycin C-treated splenocytes (Table I, experiment 1) or in vivo GaMD-activated irradiated splenocytes (Table I, experiment 2) were used as stimulator cells, both the Mls^c determinants detected by Mls^a AKR/J T cells and the Mls^a determinants detected by Mls^c C3H/HeJ T cells on C58/J stimulator cells elicited very potent levels of responder cell proliferation that were approximately equivalent. Consistent with the finding that both Mls^a and Mls^c determinants were expressed on C58/J splenocytes was the observation that H-2-compatible Mls^{a,c} (AKR × C3H)F$_1$ responder cells did not proliferate at a level much above background to these stimulator cells, a reflection of T cell genetic self-tolerance to these non-MHC moieties. Interestingly, the Mls^a and Mls^c determinants detected on the CE/J stimulator cells in this primary MLR also elicited equally vigorous cell division with appropriate responder populations. Thus, the Mls^a determinant is not unique in its ability to provide a very powerful T cell stimulus. The Mls^c gene products, at least in the context of C58/J and CE/J non-MHC backgrounds, can also induce very high levels of responder cell proliferation in vitro that approach those commonly associated with response to Mls^a.

In agreement with the reports that an Mls^a-specific hybridoma (34) and Mls^a- (but not Mls^c-) specific T cell clones (10, 11) proliferated to MA/My stimulator cells, Mls^c C3H/HeJ T cells that should recognize the Mls^a determinant showed a substantial level of cell division when cultured with normal or GaMD-activated MA/My stimulator cells (Table I, experiments 1 and 2). However, the level of responder cell proliferation elicited across an Mls^a barrier by MA/My stimulator cells was relatively low when compared with other Mls^a-bearing stimulator cells. MA/My splenocytes in these experiments also consistently brought about a low level of Mls^a AKR/J and Mls^{a,c} (AKR × C3H)F$_1$ T cell proliferation that was above background. MA/My stimulator cells also elicited proliferation

TABLE I

Analysis of Mls[a] and Mls[c] stimulatory capacity of splenocytes from selected H-2[k] inbred mouse strains

Stimulator Cells[a]		Responder Cells[b]						
Strain	Mls	AKR/J (Mls[c] MLR)		C3H/HeJ (Mls[a] MLR)		(AKR × C3H)F$_1$		CByD2F$_1$ (H-2 MLR)
		Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1
AKR/J	a	5,412[c]	3,181	157,908	320,764	4,946	3,668	79,955
C3H/HeJ	c	17,471	84,035	2,864	9,925	5,413	6,916	104,228
(AKR × C3H)F$_1$	a, c	13,606	11,897	181,983	335,712	5,677	3,192	NT[d]
CBA/J	a, c	16,467	27,355	200,133	346,568	4,204	7,217	97,799
C58/J	a, c	174,834	306,519	173,737	290,487	7,986	8,111	99,620
CE/J	a, c	115,814	NT	97,485	NT	4,779	NT	79,632
MA/My	a	13,310	11,974	33,209	79,641	12,101	11,020	97,496
RF/J	a, c	10,391	6,923	234,789	282,757	4,813	3,362	97,682
C57BL/10	b	101,230	90,130	49,376	61,009	76,674	60,267	87,442
CByD2F$_1$	a, c	NT	NT	NT	NT	NT	NT	7,375

[a] Stimulator cells were normal (nonactivated) splenocytes treated with mitomycin C (experiment 1) or GaMD-treated splenocytes that were irradiated with 3000 r (experiment 2).

[b] Responder cells were nylon wool-purified splenic T cells (3×10^5/well).

[c] Values are cpm of mean arithmetic [^{3}H]TdR uptake of quadruplicate cultures.

[d] Not tested.

of Mls^{a,c} CBA/J T above background levels (data not shown). These results indicate that, besides encoding an Mls^a determinant, MA/My stimulator cells may also express a poorly stimulatory non-Mls^{a,c} non-MHC Ag.

Like C58/J and CE/J, RF/J stimulator cells were previously determined by the use of Mls-specific T cell clones to present both Mls^a and Mls^c determinants (10, 11). In the primary MLR analysis conducted here, the vigorous proliferation of Mls^c C3H/HeJ T cells triggered by normal mitomycin C-treated or GaMD-activated RF/J stimulators was consistent with the expression of the highly stimulatory Mls^a epitope on these splenocytes (Table I, experiments 1 and 2), in agreement with the results from another laboratory (27). However, the Mls^a AKR/J T cells that should recognize the Mls^c determinant on RF/J stimulator cells showed an extremely low level of proliferation only slightly above background in this and other experiments not shown. Thus an additional experiment was performed to provide confirmation that RF/J stimulator cells were genotypically Mls^{a,c}.

If the genome of parental RF/J mice encodes both of these distinct Mls specificities, T cells obtained from this strain of mouse should not proliferate to Mls^a or Mls^c determinants presented on H-2-compatible prototypic stimulator cells. Furthermore, the F$_1$ animal obtained by crossing B10.BR (H-2^k, Mls^b) mice that are responsive to both Mls^a and Mls^c determinants with RF/J mice should also be nonresponsive to Mls^a and Mls^c determinants because of genetic self-tolerance. When this experiment was performed (Table II), the lack of responsiveness of RF/J and (B10.BR × RF/J)F$_1$ T cells to the prototypic Mls^a, Mls^c, and Mls^{a,c}-bearing stimulator cells was consistent with RF/J mice encoding both Mls^a and Mls^c in their genome. Thus the extremely low amount of Mls^a AKR/J T cell proliferation directed toward RF/J stimulator cells (Table I) is most likely generated by recognition of the very poorly presented Mls^c determinants. This defective ability of RF/J to present Mls^c accounts for the previous report that RF/J and AKR/J are mutually nonstimulatory in a primary MLR (27). However, despite profound differences in Mls-stimulatory capacity, each of these H-2^k stimulator cells effectively presented its allogeneic MHC determinants (Table I, experiment 1). Thus no generalized defect in the presentation of alloantigens existed for any of these stimulator splenocytes.

In time course analyses, across an Mls^a barrier (Fig. 1, *left*), it was clear that throughout the culture period C58/J, CE/J, and RF/J stimulator cells elicited the same high level of responder proliferation as prototypic AKR/J and CBA/J stimulator splenocytes. Consistent with previous experiments, MA/My stimulator cells provoked substantially reduced T cell proliferation across the same non-MHC difference during this time frame. When proliferation across an Mls^c barrier (Fig. 1, *right*) was subsequently examined in a separate experiment, C58/J and CE/J splenocytes elicited substantially better proliferation during the periods of optimal stimulation than did prototypic C3H/HeJ stimulator cells. In other experiments (Table I; Fig. 2), when the Mls^a and Mls^c stimulatory capacities of C58/J and CE/J were compared at the same time, these stimulator cells were found to provoke similarly high levels of T cell proliferation across both these non-MHC barriers. RF/J splenocytes elicited undetectable to barely detectable levels of responder proliferation across an Mls^c difference in this time course study. Thus the examination of nonprototypic Mls^a and/or Mls^c-bearing splenic stimulator cells reveals widely varying differences in the capacity to present both non-MHC moieties. These observations are not compatible with the usually stated position (2–7, 10, 14, 15, 18, 21–35) that equates Mls^a only to highly stimulatory and Mls^c only to poorly/intermediately stimulatory determinants in a primary MLR.

Stimulator cell capacity to elicit different levels of responder proliferation across an Mls^a or Mls^c difference correlates with T cell growth factor activity elaborated in supernatants. Although proliferation assays are frequently used to quantitate T cell activation after exposure to various stimuli, the release of cytokines such as IL-2 also serves as a sensitive measure of T cell activation (40). In the primary MLR, T cell costimulatory molecules, IL-2 and IL-4, are produced and can be detected in the supernatants by bioassay with appropriate indicator cell lines (41).

However, the elaboration of substantial levels of T cell-derived IL-2 has been detected in the absence of proliferation to certain alloantigen-presenting cells of B cell origin (42). Thus, the amount of T cell proliferation and the level of cytokine production may not always parallel one another in every experimental system. Accordingly, the relationship between the differing levels of responder cell proliferation elicited by the various Mls^a and/or Mls^c

TABLE II

The genome of RF/J mice encodes both Mls^a and Mls^c determinants

Stimulator Cells[a]				Responder Cells[b]		
Strain	H-2	Mls	Cell No. × 10^{-5}	B10.BR (H-2^k, Mls^b)	RF/J (H-2^k Mls^{a,c})	(B10.BR × RF/J) F_1 (H-2^k, Mls^{b,a,c})
Syngeneic	k		1.5	1.339[c]	3.114	2.558
			3	1.046	3.308	4.410
			6	1.197	4.622	3.861
AKR/J	k	a	1.5	116.571	2.490	2.782
			3	147.943	4.894	4.290
			6	124.984	4.722	3.659
C3H/HeJ	k	c	1.5	18.645	5.239	4.684
			3	26.502	6.515	7.757
			6	40.880	9.329	7.656
CBA/J	k	a, c	1.5	124.608	3.925	3.839
			3	161.543	5.365	5.602
			6	141.495	7.115	5.407
B10.D2	d	b	1.5	46.589	76.653	82.169

[a] Stimulator cells were GaMD-treated splenocytes that were irradiated with 3000 r.
[b] See legend to Table I.
[c] See legend to Table I.

presenting cells and relative amount of T cell growth factor activity (IL-2, IL-4) produced in these primary MLR was explored.

Figure 2*A* shows the maximal proliferation values of AKR/J (Mls^c MLR) or C3H/HeJ (Mls^a MLR) T cells obtained in a dose response study by using varying numbers (1 to 6 × 10^{-5}) of mitomycin C-treated, Mls-disparate splenic stimulator cells; the MLR was harvested at 108 h after the initiation of culture. The relative level of responder cell proliferation across an Mls^a or Mls^c barrier triggered by each stimulator population was consistent with the results presented above. In Figure 2*B*, the proliferation of the IL-2- and IL-4-sensitive HT-2 cell line in response to 48-h supernatants (1 to 4 dilution) from each corresponding MLR is displayed. The level of HT-2 indicator cell proliferation in response to the cytokine-containing supernatants mirrored rather precisely the level of T cell proliferation observed in the original Mls^a- or Mls^c-defined primary MLR. Of particular interest was the RF/J stimulator population that elicited virtually no responder cell division or T cell growth factor activity (IL-2, IL-4) across an Mls^c barrier (in contrast to highly stimulatory CE/J or C58/J splenocytes). However, RF/J stimulator cells were able to provoke very high levels of T cell proliferation and cytokine release across an Mls^a difference. These observations suggest that there may be unique sets of non-MHC regulatory controls that exert separate influences on the presentation of Mls^a or Mls^c determinants expressed by the same stimulator population.

A single dominant non-MHC gene regulates the presentation of Mls^c in the super-stimulatory form. Presentation of Mls^c by the splenocytes from parental BALB.K and C3H/HeJ mice, their F_1, first backcross, and F_2 progeny in a previous study from this laboratory suggested that, besides the well documented role of the MHC in regulating Mls^c stimulatory capacity, multiple non-MHC genes (8) also exert an important influence.

In this investigation, the study of non-MHC genetic influences that regulate the presentation of Mls^c determinants is extended by utilizing parental strains in the segregation analysis that express the super-stimulatory form of Mls^c (C58/J) or poorly present this non-MHC moiety (BALB.K). When the (BALB.K × C58/J)F_1 animals were generated and their mitomycin C-treated normal splenocytes were used to present Mls^c determinants in a primary MLR, these F_1 stimulator cells consistently approximated the C58/J parental splenocytes over a range of cell concentrations in triggering very high levels of H-2-compatible T cell proliferation (Fig. 3, *upper left*). Thus the super-stimulatory Mls^c presenting capacity of the (BALB.K × C58/J)F_1 splenocytes differed substantially from the previously studied Mls^c stimulatory capacity of (BALB.K × C3H/HeJ)F_1 splenocytes that was intermediate between BALB.K and C3H/HeJ parental stimulator cells (8).

In a similar experiment, CE/J (Mls^c super-stimulatory) mice were crossed with RF/J mice that were the least capable of any H-2^k strain examined so far in regard to Mls^c presenting ability. Again, mitomycin C-treated splenocytes from the (RF/J × CE/J)F_1 animals closely resembled the Mls^c super-stimulatory parent, CE/J, in their capacity to present this non-MHC determinant (Fig. 3, *lower left*). Mendelian genetic theory predicts that no intermediate phenotype will occur when the inheritance pattern of a trait is controlled by a single dominant locus. Thus, the results of these two experiments were compatible with presentation of the super-stimulatory form of Mls^c being regulated by a single dominant non-MHC gene. In these experiments, although both BALB.K and RF/J stimulator cells were poor presenters of Mls^c, they showed no impairment in their capacity to present MHC determinants in a primary MLR (Fig. 3, *upper* and *lower right*). Splenocytes from an F_1 animal obtained by crossing an Mls^a super-stimulatory strain, CBA/J, and an Mls^a poorly stimulatory strain, MA/My, also more closely resembled the super-stimulatory parent in eliciting vigorous levels of C3H/HeJ T cell proliferation across an Mls^a difference (data not shown). However, the issue of the dominance of the super-stimulatory form of Mls^a in these (CBA/J × MA/My)F_1 stimulator cells is complicated by the observation that MA/My may lack a component of the Mls^a determinant usually expressed by prototype strains (see below).

To test further the hypothesis that a single non-MHC regulatory gene controlled the presentation of the super-

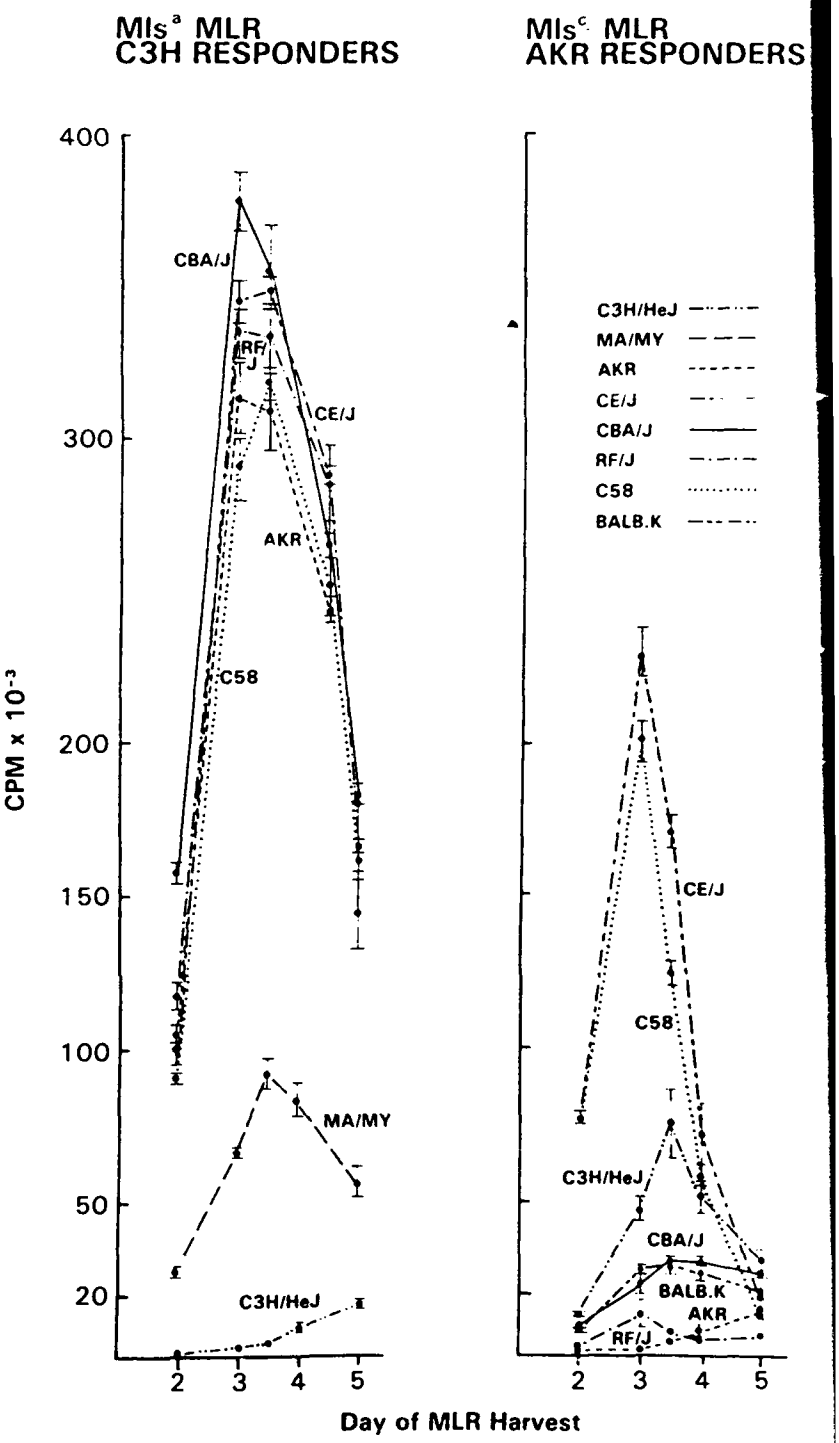

Figure 1. Time course of proliferation of responder cells to Mls^a or Mls^c determinants expressed by selected H-2^k stimulator cells. C3H/HeJ (H-2^k, Mls^c) or AKR/J (H-2^k, Mls^a) nylon wool-purified T cells (3×10^5/well) were cultured with H-2^k-compatible GaMD-treated splenocytes (3 to 6×10^5/well) in separate experiments to assess their Mls^a (*left*) or Mls^c (*right*) stimulatory capacity at 2, 3, 4, and 5 days after the MLR culture was established.

stimulatory Mls^c phenotype, (BALB.K × C58)F_1 × BALB.K first backcross and (BALB.K × C58)F_2 mice were generated. Splenocytes from these animals, after mitomycin C treatment, were individually analyzed for Mls^c presenting capacity with AKR/J (Mls^a) responder T cells in a primary MLR in comparison with stimulator cells from parental and F_1 types (Fig. 4). The results indicated that the first backcross animals segregated into two easily detectable phenotypic populations in terms of Mls^c stimulatory capacity. A total of 20 of the 45 first backcross animals (44.4%) resembled parental C58/J and F_1 animals in possessing a potent Mls^c stimulatory capacity; however, the remaining 25 animals (55.6%) demonstrated a poor Mls^c stimulatory ability that resembled the BALB.K parental type. With the 59 F_2 progeny examined, 48 animals (81.4%) most closely resembled the highly Mls^c stimulatory C58/J and F_1 mice whereas the other 11 animals (18.6%) had the poorly Mls^c stimulatory phenotype resembling the BALB.K parent. χ^2 analysis of the first backcross ($\chi^2 = 0.556$) and F_2 ($\chi^2 = 1.271$) data does not allow rejection of the hypothesis that a single gene regulates the presentation of the super-stimulatory form of the Mls^c determinant at the 5% level of significance. However, the hypothesis that two codominant genes control the expression of super-stimulatory Mls^c can easily be rejected at this level of significance by χ^2 analysis of the first backcross ($\chi^2 = 22.41$) and F_2 ($\chi^2 = 15.45$) stimulatory capacity.

The non-MHC influence responsible for the super-stimulatory form of Mls^c is exerted in splenocytes from

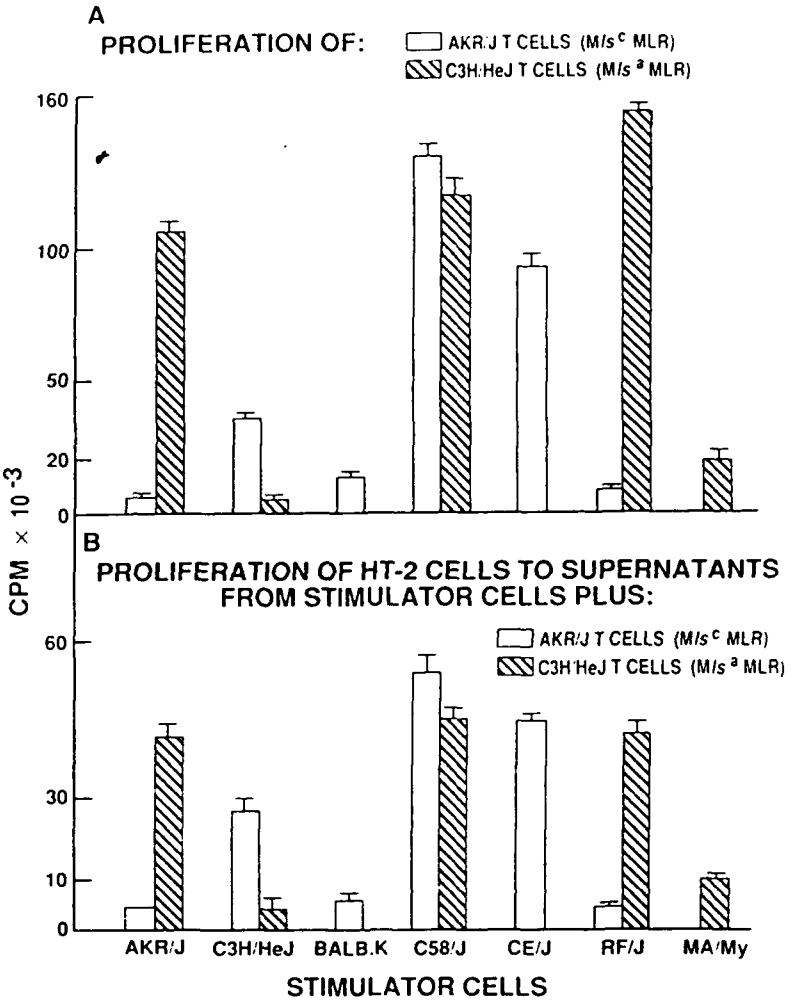

Figure 2. Comparison of the level of responder T cell proliferation elicited with the level of T cell growth factor activity elaborated in an Mls^a or Mls^c-defined MLR triggered by various H-2^k-compatible stimulator cells. AKR/J (H-2^k, Mls^a) or C3H/HeJ (H-2^k, Mls^c) nylon wool-purified T cells (3 × 10^5/well) were cultured with normal mitomycin C-treated splenocytes (1 to 6 × 10^5/well) of the stimulator strains listed; the MLR was harvested at 108 h after the culture was established, to assess maximal T cell proliferation across an Mls^a (▨) or Mls^c (□) barrier (*A*). HT-2 cells (4 × 10^3/well) were cultured for a 24-h period with the 48-h supernatants (diluted 1/4) from each of the corresponding MLR to assess T cell growth factor activity elaborated across an Mls^a (□) or Mls^c (□) difference (*B*).

XID$^+$ B cell-defective CBA/N F$_1$ male animals as well as B cell-normal CBA/N F$_1$ female littermates. Because B lymphocytes are capable of presenting Mls determinants (32, 35, 39, 43) and may be the cell type in which the non-MHC regulatory genes act, we wished to determine if the non-MHC influence that confers super-stimulatory capacity to Mls^c in the B cell-normal C58/J mice could be operative in the B cell-defective environment of xid$^+$ splenocyte stimulator cells. Because the frequency of Ig-positive splenocytes in xid$^+$ CBA/N F$_1$ male mice is approximately 40% less than that found in their phenotypically B cell-normal female F$_1$ littermates (44), male and female CBA/N F$_1$ stimulator cells were depleted of T cells by treatment with anti-Thy 1.2 + C to obtain approximately equivalent numbers of non-T Mls-presenting cells in both groups.

In agreement with a previous report (45), the xid$^+$ (CBA/N × C3H/HeJ)F$_1$ male stimulator splenocytes did not elicit convincing levels of proliferation across an Mls^c difference whereas the lymphoid cell population from phenotypically B cell-normal female F$_1$ littermates was capable of triggering T cell proliferation across this non-MHC barrier (Table III, experiment 1). In contrast, the xid$^+$ (CBA/N × C58/J)F$_1$ male stimulator cells were capable of triggering a significant level of responder cell proliferation directed toward an Mls^c disparity (Table III, experiment 2). Nevertheless, the splenocytes from the (CBA/N × C58/J)F$_1$ female mice more effectively presented both Mls^c and Mls^a determinants than stimulator cells from the B cell-defective F$_1$ male animals. It was also consistent with other reports (26) that the splenocytes from adult xid$^+$ (CBA/N × C58/J)F$_1$ male animals elicited comparable levels of proliferation at optimal cell concentrations across an MHC barrier in comparison with the B cell-normal F$_1$ female littermates.

In regard to the presentation of Mls^a determinants by the xid$^+$ (CBA/N × C58/J)F$_1$ stimulator cells, the splenocytes from the male F$_1$ animals were less effective stimulator cells across this barrier than they were across the Mls^c barrier (Table III, experiment 2). However, previous experiments (Table I; Figs. 1 and 2) have suggested that independent non-MHC regulation of Mls^a and Mls^c determinants exists for stimulator cells presenting both of these specificities simultaneously. Consequently, it is possible that non-MHC genes in the C58/J background that may control or upregulate the presentation of the Mls^a may be less capable of penetrating or functioning in the xid$^+$ environment than is the non-MHC gene that enhances the presentation of super-stimulatory Mls^c.

Thus this series of experiments indicated that the non-MHC genetic control that regulated the super-stimulatory form of Mls^c determinants expressed by B cell-normal presenting cells was able to exert its influence in the B cell-defective environment of xid$^+$ non-T cell stimulator cells. However, this potent non-MHC genetic influence could not totally overcome the xid defect in the presentation of the Mls^c specificity.

Parental, non-prototypic, poorly stimulatory Mls^c

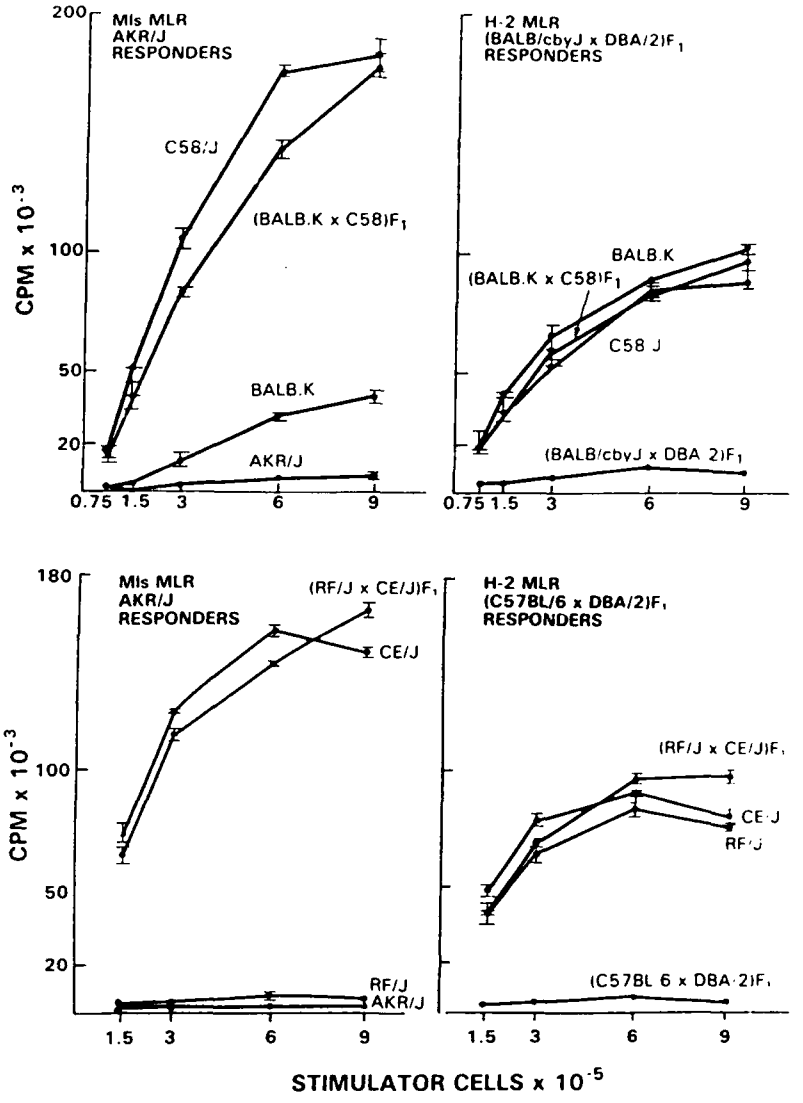

Figure 3. Comparison of Mls and H-2 stimulatory capacity of splenocytes from BALB.K, C58/J, and their F_1 as well as RF/J, CE/J, and their F_1. Nylon wool-purified splenic T cells (3×10^5/well) from AKR/J (H-2^k, Mls^a) mice were utilized to detect the Mls^c-stimulatory capacity of normal mitomycin C-treated splenocytes from C58/J (H-2^k, Mls^{a,c}), BALB.K (H-2^k, Mls^c), and their F_1 (*upper left*) or CE/J (H-2^k, Mls^{a,c}), RF/J (H-2^k, Mls^{a,c}), and their F_1 (*lower left*). Similarly purified (BALB/c × DBA/2)F_1 (H-2^d, Mls^{a,c}) or (C57BL/6 × DBA/2)F_1 (H-$2^{b/d}$, Mls^{a,c}) splenic T cells (3×10^5/well) were utilized, respectively, to detect the H-2 stimulatory capacity of C58/J, BALB.K, and their F_1 (*upper right*) or CE/J, RF/J, and their F_1 (*lower right*).

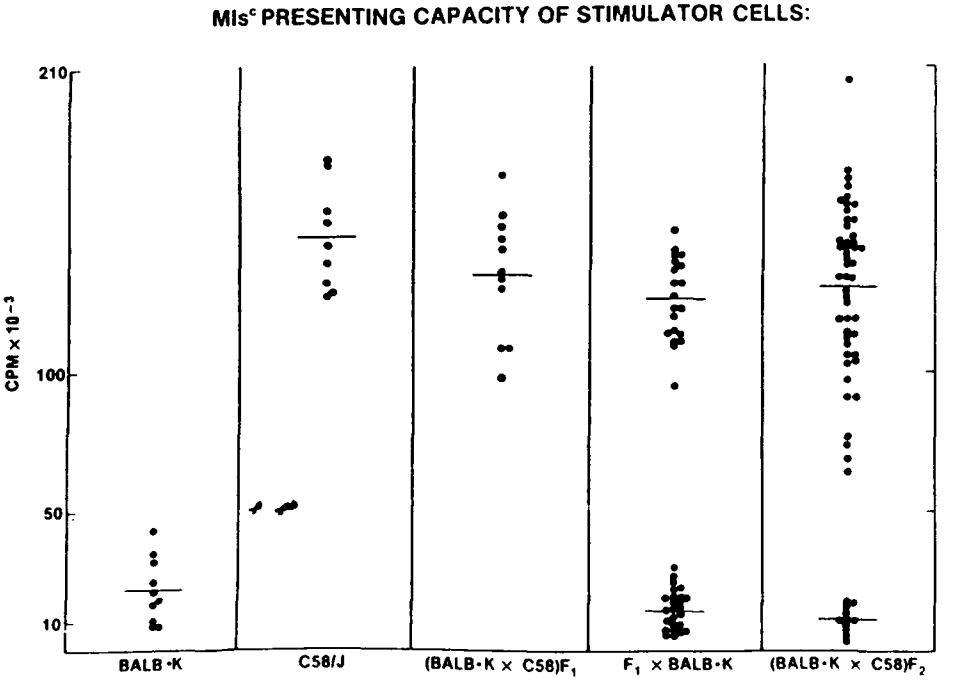

Figure 4. Comparison of Mls^c-stimulatory capacity of splenocytes from parental BALB.K, C58/J, their F_1, F_2, and first backcross. Nylon wool-purified AKR/J (H-2^k, Mls^a) splenic T cells (3×10^5/well) were cultured with normal mitomycin C-treated splenocytes (6×10^5/well) from C58/J (H-2^k, Mls^{a,c}), BALB.K (H-2^k, Mls^c), (BALB.K × C58/J)F^1, (F_1 × BALB.K) first backcross, and (BALB.K × C58/J)F_2 mice to detect their Mls^c stimulatory capacity. The MLR cultures were harvested after 72 h. The horizontal lines indicate the mean cpm value of T cell proliferation elicited by each stimulator cell group.

TABLE III

Non-MHC regulatory genes exert their influence on the presentation of Mls^c in splenocytes from XID$^+$ B cell-defective CBA/N F_1 male and phenotypically B cell-normal CBA/N F_1 female mice

Expt. No.	Stimulator Cells[a]		Responder Cells[b]		
	Strain	Cell No. × 10^{-5}	AKR/J (Mls^c MLR)	C3H/HeJ (Mls^a MLR)	B6D2F_1 (H-2 MLR)
1	Syngeneic	2	5.187[c]		
		4	8.785		
		6	11.874		
	(CBA/N × C3H)F_1 (male)	2	4.968		
		4	9.109		
		6	15.291		
	(CBA/N × C3H)F_1 (female)	2	20.748		
		4	40.051		
		6	49.692		
2	Syngeneic	3	2.066	2.495	1.612
		6	2.728	2.379	1.118
	(CBA/N × C58)F_1 (male)	3	28.165	7.355	39.303
		6	45.084	12.851	26.336
	(CBA/N × C58)F_1 (female)	3	100.859	37.290	46.968
		6	151.971	98.781	41.231

[a] Stimulator cells were normal splenocytes, treated first with anti-Thy-1.2 + C and then with mitomycin C.
[b] See legend to Table I.
[c] See legend to Table I.

mouse strains, BALB/c and DBA/2, generate self-tolerance in F_1 progeny to the super-stimulatory form of Mls^c. Several laboratories have demonstrated that multiple unlinked structural genes contribute to the Mls^c genotype in prototypic C3H mice (13–15). Indeed, there is some evidence that different mouse strains may have different numbers of genes associated with Mls^c phenomena (15). Therefore, it cannot be assumed that a given mouse strain currently classified as Mls^c will encode all of the structural genes associated with the Mls^c of prototypic C3H. Accordingly, it was of interest to determine if the Mls^c genetic elements amplified by a single non-MHC gene to super-stimulatory status would also be encoded in the genome of Mls^c nonprototypic and poorly stimulatory (8) BALB/c and DBA/2 mice. To address this point, the proliferation of T cells derived from (AKR/J × B10.D2)F_1 mice that are fully responsive to prototypic or super-stimulatory Mls^c expressed on C3H/HeJ and C58/J mitomycin C-treated splenocytes, respectively, was compared with the proliferation of H-$2^{k/d}$-compatible (AKR/J × BALB/c)F_1 and (AKR/J × DBA/2)F_1 responder cells to these stimulator populations (Table IV, experiment 1).

If BALB/c and DBA/2 encode the genetic component of C3H Mls^c that is amplified on C58/J presenting cells, the T cells obtained from the latter two groups of F_1 animals should be unresponsive both to H-2^k-compatible C3H/HeJ and C58/J stimulatory cell because of genetic self-tolerance. Indeed, in this primary MLR, a total lack of responsiveness to C3H/HeJ and C58/J mitomycin C treated splenocytes was observed with (AKR/J × BALB/c)F_1 and (AKR/J × DBA/2)F_1 T cells. However, they did proliferate vigorously to H-2^s-different SJL/J third party stimulator cells. Self-tolerance to super-stimulatory Mls^c was confirmed in another experiment (Table IV, experiment 2), in which the two (AKR/J × C58/J)F_1 × B10.BR backcross animals that typed as super-stimulatory Mls^c were nonstimulatory for (AKR/J

TABLE IV

F_1 animals generated from Mls^c nonprototypic BALB/c or DBA/2 parental mouse strains are self-tolerant to the super stimulatory form of Mls^c

Expt. No.	Stimulator Cells[a]	Responder Cells[b]		
		(AKR × B10.D2)F_1	(AKR × BALB/c)F_1	(AKR × DBA/2)F_1
1	Syngeneic	5.596[c]	5.682	5.659
	C3H/HeJ	51.480	5.923	10.539
	C58/J	222.940	7.505	7.752
	SJL/J	148.027	145.246	111.894
2	Syngeneic	3.085	4.442	
	C3H/HeJ	29.888	3.823	
	C58/J	136.245	6.281	
	(B10.BR × C58)F_1	130.018	4.015	
	(AKR × C58)F_1 × B10.BR			
	1	4.904	3.322	
	2	7.356	7.688	
	3	123.238	5.916	
	4	3.964	3.374	
	5	122.516	3.342	
	6	2.424	1.468	
	C57BL/10	51.939	51.247	

[a] Stimulator cells were mitomycin C-treated normal splenocytes.
[b] See legend to Table I.
[c] See legend to Table I.

× BALB/c)F_1 T cells.

The finding that the BALB non-MHC background (that is associated with poor presentation (8) of Mls^c by both Ia^k- and Ia^d-bearing stimulator cells) likely encodes the Mls^c genetic element upregulated on C58/J stimulator cells strengthens the previous contention that it was the action of a single non-MHC regulatory gene in (BALB.K × C58/J)F_1, (BALB.K × C58/J)F_1 × BALB.K backcross and (BALB.K × C58/J)F_2 animals (Fig. 4) that controlled the expression of the super-stimulatory form of Mls^c. In addition, although CBA/J splenocytes usually present Mls^c more poorly (8) than prototypic C3H/HeJ, T cells from the former strain were also found to be unresponsive to Mls^c super-stimulatory C58/J or CE/J splenocytes (data not shown). Taken together, these results suggest that the Mls^c genetic element amplified on C58/J presenting cells by a single non-MHC gene is distributed among prototypic and nonprototypic Mls^c-bearing mouse strains.

Mls^a and Mls^c prototypic mouse strains may express an additional Mls specificity. To confirm earlier observations about the Mls composition of RF/J, CE/J, C58/J, and MA/My mouse strains, T cells were isolated from each and cultured with H-2^k-compatible Mls^a prototypic AKR/J and Mls^c prototypic C3H/HeJ stimulator cells (Table V).

The lack of proliferation of RF/J and CE/J T cells to prototypic Mls^a and Mls^c stimulator cells (Table V, experiments 1 and 2) was consistent with the primary MLR data previously presented (Tables I and II) and the reports using Mls^a-specific clones and Mls^c-specific clones (10, 11) that indicated RF/J and CE/J splenocytes expressed both Mls^a and Mls^c specificities. However, the substantial level of proliferation of C58/J and MA/My T cells to the Mls prototypic stimulator cells with which they were supposedly Mls and H-2 compatible suggested an unanticipated complexity in understanding the Mls genotypes not only of these two responder cells but also the AKR/J (Mls^a) and C3H/HeJ (Mls^c) prototypic stimulator cells (Table V, experiments 3 and 4).

Because Mls^a-specific clones and Mls^c-specific clones (10, 11) as well as Mls^a (AKR/J) and Mls^c (C3H/HeJ), but not Mls^{a,c} ((AKR × C3H)F_1)), unprimed T cells (Table I) proliferated vigorously on C58/J stimulator cells, it seemed most likely that both Mls^a and Mls^c determinants were expressed on C58/J splenocytes. Other Mls^{a,c} F_1 T cells, (AKR/J × BALB/c)F_1 and (AKR/J × DBA/2)F_1 (Table IV) as well as CBA/J (data not shown), also did not respond to the potent non-MHC specificities presented by C58/J lymphoid cells. Thus the convincing proliferation of Mls^{a,c} C58/J T cells to Mls^a and Mls^c prototypic stimulator cells could represent the recognition of a newly defined Mls specificity.

The proliferation of Mls^c C3H/HeJ heterogeneous T cells (Table I) and cloned Mls^a-specific (but not cloned Mls^c-specific) T cells (10, 11) in response to MA/My stimulator cells was consistent with the expression of Mls^a but not Mls^c moieties on MA/My splenocytes. The response of Mls^a MA/My T cells to Mls^a AKR/J stimulator cells may indicate that the prototypic Mls^a determinant contains a component not shared with MA/My.

Mitomycin C-treated normal splenocytes (like the GaMD-activated irradiated stimulator cells used in Table V) also elicited these same unexpected C58/J and MA/My T cell non-MHC MLR proliferative responses (data not shown).

DISCUSSION

A commonly held contention in many previously published Mls studies (2–7, 10, 14, 15, 18, 21–35) is that the unprimed heterogenous T cell response to the Mls^c determinants is significantly less vigorous than it is to the Mls^a gene products. This view is based, however, only on the analysis of stimulator cells from the original prototypic Mls^c-bearing mouse strains, C3H and A/J, identified by Festenstein (2, 3) over a decade ago. The primary MLR-stimulatory capacity of Mls^c and Mls^a determinants expressed on strains of mice recently identified to encode these non-MHC determinants, by use of Mls-specific T cell clones (10, 11), has not been extensively explored.

Because non-MHC (in addition to MHC) genes can cause substantial variation in Mls-presenting ability among inbred mouse strains (8), we questioned whether the accepted characterization of Mls^c as a weakly or intermediately stimulatory or Mls^a as highly stimulatory would hold true upon closer scrutiny of nonprototypic Mls-bearing mouse strains.

Across an Mls^c barrier there was an impressive gradient of MLR stimulatory potential displayed with splenocytes obtained from the nonprototypic mouse strains. At one extreme, RF/J stimulator cells were virtually incapable of presenting this non-MHC specificity in a primary

TABLE V

Analysis of RF/J, CE/J, C58/J, and MA/MyJ T cell responsiveness to prototypic Mls^a and Mls^c stimulator cells

Expt. No.	Responder Cells[a]		Stimulator Cells[b]			
	Strain	Mls	Syngeneic	AKR/J (Mls^a)	C3H (Mls^c)	B10.D2 (H-2^d)
1	B10.BR	b	4,435[c]	105,178	26,089	64,802
	RF/J	a, c	7,600	6,551	9,272	85,968
2	B10.BR	b	4,711	182,069	25,883	61,676
	CE/J	a, c	10,851	7,928	5,858	99,749
3	B10.BR	b	2,949	225,725	27,286	106,212
	C58/J	a, c	4,959	44,261[d]	30,926	73,797
4	B10.BR	b	2,735	164,582	65,427	78,961
	MA/MyJ	a	4,852	54,321	72,447	77,498

[a] Responder cells were nylon wool-purified splenic T cells.
[b] Stimulator cells were GaMD-treated splenocytes that were irradiated with 3000 r.
[c] See legend to Table I.
[d] Underlining indicates the unanticipated proliferation of responder cells to the Mls prototypic stimulator cell that shares the Mls specificity with the responder.

MLR as measured by responder T cell proliferation or in vitro elaboration of T cell growth factor activity (IL-2, IL-4). In marked contrast, C58/J and CE/J splenocytes were capable of triggering extremely high levels of responder cell division and cytokine production in an Mls^c-defined primary MLR. The amount of T cell proliferation and T cell growth factor activity elicited by the latter two stimulator populations across an Mls^c barrier reached levels commonly associated with the Mls^a determinant. Prototypic C3H/HeJ stimulator cells fell in between very poorly stimulatory RF/J and super-stimulatory C58/J and CE/J in Mls^c presenting capacity.

Across the "historic" super-stimulatory Mls^a barrier, H-2^k MA/My stimulator cells had a rather modest capacity to stimulate H-2-compatible T cell proliferation or T cell growth factor production when compared with prototypic Mls^a-bearing AKR/J and CBA/J or less well studied C58/J and CE/J splenocytes. Among H-2^d mouse strains, we previously observed that LT/ChReSv splenocytes presented Mls^a determinants less effectively than prototypic DBA/2 stimulator cells (8).

Thus for both an Mls^a difference and an Mls^a difference, these Mls nonprototypic mouse strains provided important examples of Mls stimulatory potential (as a result of the influence of the non-MHC environment) that was in sharp contrast to that usually associated with the AKR/J (Mls^a) and C3H/HeJ (Mls^c) prototypic strains.

Although the T cell repertoire appears to contain fewer cells responsive to Mls^a (Vβ3$^+$) than Mls^a (Vβ6$^+$ and Vβ8.1$^+$) (16–18, 19), equivalent levels of responder proliferation (Table I; Fig. 2) and cytokine production (Fig. 2) were elicited by super-stimulatory Mls^a and super-stimulatory Mls^c-bearing splenocytes. Thus one might speculate that the non-MHC gene that controls the super-stimulatory forms of Mls^a may act by increasing the intensity of this signal delivered to Vβ3$^+$ T cells and thereby allow very rapid expansion of this responder population.

Clearly diverse genetic regulatory mechanisms are operating to control the in vitro presentation of Mls^c in these different non-MHC environments. This variety of non-MHC genetic influences is exemplified by the action of: a) multiple non-MHC IR genes that control the presentation of the weakly to intermediately stimulatory Mls^c determinants in the first backcross and F_2 progeny derived from BALB.K and C3H/HeJ parental strains (8), b) a dominant single non-MHC gene that controls the presentation of the super-stimulatory form of Mls^c present on C58/J (and probably CE/J) stimulator cells (Fig. 4), and c) the recessive non-H-2-determined genetic influence that brings about the virtual elimination of Mls^c-stimulating ability for RF/J splenocytes (Fig. 3).

Differing, but not necessarily mutually exclusive, mechanisms might account for this non-MHC-regulated broad spectrum of Mls^c-stimulatory capacity for presenting cells with fully permissive H-2 haplotypes. Because identical, or at least highly cross-reactive, immunodominant non-H-2 Ag capable of stimulating the generation of cytotoxic lymphocytes were mapped to independently segregating loci in different recombinant inbred mouse strains (46), the possibility may exist that the genes encoding Mls^c or Mls^a determinants also exhibit a certain "mobility" and are located on different chromosomes in different mouse strains. If this is true, the radically differing non-MHC milieu of enhancer, promoter, or modifier genes (associated with different chromosomal locations) that influence the stimulation of transcription and tissue-specific gene expression could cause Mls-presenting capacity to vary remarkably among genetically disparate strains of H-2-compatible mice. Alternatively, if the alleles of a given Mls gene uniformly map to the same chromosomal location for all strains of mice, local variation in the structure or positioning of enhancer, promoter, or (even nonlinked) modifier sequences could bring about differences in expression and thus presentation of these non-MHC specificities.

In mice that have a permissive H-2 but low or virtually undetectable Mls^c stimulatory capacity, other self-Ag peptides might be present that have a higher affinity for the binding site of class II MHC molecules and thus effectively compete out the the presentation of Mls Ag. Such competition for interaction with Ia molecules has been described for immunogenic hen egg lysozyme peptides (47). Furthermore, non-MHC-regulated genetic differences that in vitro could bring about inappropriate shedding or processing of Mls moieties might also contribute to the poor Mls-presenting capacity that is reproducibly associated with certain mouse strains having fully permissive H-2 haplotypes. The mixing, in a primary MLR, of stimulator cells that have a permissive MHC yet elicit extremely low levels of response across an Mls^c barrier with H-2-compatible stimulators that trigger extremely high levels of T cell proliferation across this non-MHC difference, e.g., RF/J with CE/J, never gave an indication that the induction of suppressor cells played a role in eliminating the response to Mls^c determinants (data not shown).

Three other laboratories have addressed the issue of the genetic control of Mls^c determinants by using backcross analysis (13–15). Click and Adelmann (13) and Pullen et al. (14) concluded that three independently segregating loci control phenomena associated with prototypic Mls^c of the C3H mouse strain. However, Abe and Hodes (15) have presented evidence that at least two genes are involved in the expression of Mls^c encoded in C3H/HeJ mice. Interestingly, the latter investigators also found that the expression of Mls^c by CBA/J stimulator cells may be regulated by a single gene (15).

In contrast to those three studies, the segregation analysis conducted in the present as well as the previous (8) investigation from this laboratory, dealing with non-MHC regulation of Mls^c, has utilized first backcross and F_2 animals derived from parental strains that both encode Mls^c structural genes (with the exception of Table IV, experiment 2). The protocols for the experiments were designed in this way in order to observe more clearly the action of non-MHC regulatory influence on the Mls^c gene products by minimizing structural gene dose effects that might be encountered in Mls^c heterozygotes. However, it is not known for certain if RF/J and CE/J mice as well as C58/J, BALB.K, their F_1, F_2, and first backcross (Fig. 4) each encode an identical number of Mls^c structural genes, located at precisely the same chromosomal locations as in the C3H prototype strain. Nevertheless, the existence of a single dominant C58/J non-MHC regulatory gene that controls presentation/expression of super-stimulatory Mls^a provides the most economical explanation for a) the dominance of the super-stimulatory Mls^a

phenotype in (Mls^c poorly stimulatory × Mls^c super-stimulatory)F_1 animals (Fig. 3), b) the χ^2 analysis of Mls^c stimulatory potential of splenocytes from (BALB.K × C58)F_1 × BALB.K and (BALB.K × C58)F_2 progeny (Fig. 4), and c) the observation that F_1 animals generated from Mls^c poorly stimulatory and nonprototypic (8) BALB/c mice were fully self-tolerant to the super-stimulatory form of Mls^c (Table IV).

The potency of the enhancing effect of the single non-MHC gene encoded in the C58/J background on Mls^c presentation is further demonstrated by the augmented and easily detected capacity of B cell-defective xid$^+$ (CBA/N × C58/J)F_1 male non-T splenocytes to present this non-H-2 moiety in comparison with xid$^+$ (CBA/N × C3H/HeJ)F_1 male stimulator cells (Table III).

Although C58/J and CE/J stimulator cells provide extremely interesting examples of non-MHC-controlled enhancement of a cell-surface structure, further study of RF/J stimulator cells that also possess a permissive H-2 haplotype yet fail to present effectively their encoded Mls^c determinants may also yield valuable information about negative pathways of Mls gene regulation. Consistent with the classification of RF/J as Mls^{a,c}, RF/J and (B10.BR × RF/J)F_1 T cells were unresponsive to Mls^a prototypic AKR/J, Mls^c prototypic C3H/HeJ, and Mls^{a,c} prototypic CBA/J splenocytes in a primary MLR (Table II). However, we also observed some variation from experiment to experiment in the response of RF/J and (B10.BR × RF/J)F_1 T cells to H-2^k and Mls^{a,c}-compatible CE/J and C58/J stimulator cells (data not shown). The occasional low level responses to the latter stimulator cells that express the super-stimulatory form of Mls^c might mean that RF/J mice contained low levels of Vβ3$^+$ T cells. Interestingly, Pullen et al. (14) have noted a higher number of Vβ3$^+$ T cells among Mls-2^{a+} AKXD RI strains that were the poorest presenters of this non-MHC specificity.

Primary non-MHC MLR proliferative results with T cells from Mls nonprototypic mouse strains suggest a possible need for further revision of current Mls concepts.

First, the positive response of MA/My T cells to H-2^k, Mls^a-compatible (and prototypic) AKR/J stimulator cells was particularly intriguing. Although the Mls^a gene product was originally reported to be encoded by a single gene (48), we are currently investigating the possiblity that the prototypic Mls^a-defined MLR (B10.BR or C3H/HeJ anti-AKR/J) can be further delineated into responses to several non-MHC specificities: a) one shared by MA/My and AKR/J and b) the other present only on AKR/J stimulator cells and recognized by MA/My T cells. Based on the study of the distribution of Mls^a in (BALB/c × DBA/2)F_2 animals, other investigators previously proposed that Mls^a is encoded by two separate loci (12).

Second, the substantial C58/J (H-2^k, Mls^{a,c}) primary T cell response to H-2-compatible AKR/J (Mls^a) and C3H/HeJ (Mls^c) splenocytes was also unanticipated. Interestingly, the specificity recognized by C58/J T cells is very widely distributed among inbred mouse strains and is well expressed even by certain prototypic Mls^b (so-called nonstimulatory) presenting cells; this C58/J T cell response may identify a newly defined Mls moiety (J. J. Ryan, H. B. LeJeune, J. J. Mond, and F. D. Finkelman, manuscript in preparation).

Finally, the usual formulation that blankedly catagorizes Mls^a and Mls^c Ag as highly or poorly/intermediately stimulatory, respectively, obscures the critical participation of non-MHC genes in regulating the level of strain-specific expression and presentation of Mls specificities. In their most impressive modes of action, non-MHC regulatory genes can virtually eliminate or substantially enhance the presentation of these Ag to unprimed T cells. The ultimate goal of current research must be to characterize biochemically the members of the Mls "family" of molecules and clone the genes that encode them. Based on experiments incorporated in this study, we would predict that effective in vitro functional analysis of isolated Mls genes or gene products could be compromised without due consideration of potentially dramatic and different non-MHC regulatory influences that may be uniquely associated with each presenting cell population examined.

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